METHYL GREEN-COATED COLUMN FOR SEPARATION OF INORGANIC ANIONS BY ION CHROMATOGRAPHY

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SUMMARY

A polystyrene–divinylbenzene column (PRP-1) was coated with methyl green to obtain an anion-exchange column with adsorbed quaternary ammonium groups. The efficiency of the coated column was examined. Two different eluents previously applied to single-column anion chromatography with chemically bonded exchanging groups were used with only little modifications. Baseline separation for eight common anions can be achieved and water analyses, *e.g.*, mineral water, can be realized with these systems. No organic modifier is needed to obtain an efficient separation and only small amounts of dyestuff have to be added. The performance of this column is comparable to that of a chemically bonded anion-exchange column, *e.g.*, PRP-X 100. Besides efficient separation, this system provides the advantage that the column lifetime increases because the lost anion exchanging sites are replaced by the eluent which contains the dyestuff.

INTRODUCTION

One of the chromatographic separation methods for ions is ion-pair chromatography which has been developed by Eksborg *et al.*[1]. The well known effect of ion-pair formation has found wide applications in the separation of organic and also inorganic ions. Haddad and Heckenberg[2] have reviewed ion chromatography as well as "ion-pair" chromatography.

Ion interaction columns are generally produced by sorption of an organic, hydrophobic and ionic molecule onto the surface of a reversed-phase column. A charged double layer is obtained[3]. Corresponding to this principle, this technique was also called "soap chromatography"[4]. Other terms used were listed by Bidlingmeyer *et al.*[5]. Systems for the separation of inorganic anions have been developed by several authors[6–14]. Aliphatic amines and mainly quatenary ammonium salts were applied as counter ions. One of the major works on coated columns for ion chromatography is that of Duval and Fritz[15].

The retention mechanism in "ion-pair" liquid chromatography was examined by Bidlingmeyer *et al.*[5], Hung and Taylor[16] and Knox and Hartwick[17]. The authors came to different conclusions, so that no comprehensive explanation for all "ion-pair" chromatographic systems can be given.

Cassidy and Elchuk[7,10,12] produced dynamically coated and also "permanent" sorbed fixed-site ion exchangers. The systems differed only in the length of the hydrophobic hydrocarbon chain of the ion interaction reagent. A fixed-site column does not need any ion interaction reagent in the mobile phase and acts as an ion exchanger. Obviously these chromatographic systems cannot be definitively divided into "ion-pair" or ion-exchange chromatography. Cassidy and Elchuk used the more adequate term "ion interaction" chromatography. The use of dyestuffs in chromatographic separation was first described by Gnanasambandan and Freiser[18]. They employed methylene blue for the separation of aliphatic alcohols. The dye–alcohol complex formation and the partitioning equilibrium were investigated. In other studies methyl green was used for conditioning the column in ion interaction chromatography of inorganic ions to provide sharper peaks and shorter elution times, by eliminating interaction with the basis material[11]

Brilliant green as the stationary phase on a chemically bonded ODS column and the separation of aliphatic acids on this column was described by DiNunzio and Freiser[19]. The mobile phase consisted only of organic solvents. The process was described as real "ion-pair" chromatography.

Kang[20] developed a chromatographic system with methylene blue as the counter ion for the separation of organic and inorganic anions. The separation on a chemically bonded octadecyl silica gel column with an aqueous eluent and methanol as the organic modifier showed peak tailing.

Previous studies[21] pointed out that a styrene–divinylbenzene copolymer column with chemically bonded quaternary ammonium groups (PRP-X 100) showed a loss of ion-exchange capacity. The concentration of the organic acid in the eluent had to be decreased to obtain separations comparable to those achieved on a new column. Preliminary studies showed that the ion-exchange capacity can be increased by adsorption of methyl green. The basis material of a PRP-X 100 column is a PRP-1 resin[22]. Owing to these facts it should be possible to develop a methyl green-coated column for single-column anion chromatography that possesses a performance comparable to that of a chemically bonded ion-exchange column. The same simple eluents as used for chemically bonded columns are expected to be applicable.

EXPERIMENTAL

Apparatus

The HPLC equipment consisted of a dual-head reciprocating pump (Bischoff 2200), a syringe-loading sample injector (Bischoff 7125) and a variable-wavelength UV detector (Bischoff 8201) linked to a pen recorder (Kipp-Zonen BD8 multi range) or to a computing integrator (Shimadzu C-R3A). An autosampler (Talbot ASI-3) was also used instead of a syringe-loading sample injector. The variable-loop injector with an 100-μl loop was integrated in a thermobox (Bischoff 4000) kept to room temperature. The chart speed of the recorder was 1 cm/min. For studying the detector response in the visible range a UV–VIS detector (Kratos Spectroflow 757) was used.

Reagents

2,4-Dihydroxybenzoic acid (purum, Fluka; 98%) and 4-hydroxybenzoic acid (puriss, Fluka) were used. Chloride in methyl green (Fluka, for microscopy) was replaced with iodide on an anion-exchange resin. The anion exchanger (Amberlite, Type IRA 400, counter ion Cl^-) was completely transformed into the iodide form for conversion of 100 mg methyl green in 50 ml water on a short column (8 cm × 2 cm). The final volume of the dyestuff solution was 200 ml. This column was regenerated before each conversion and had been used for 3 months.
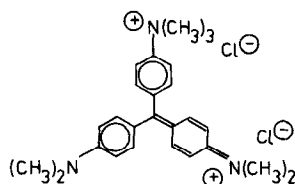
The other reagents used were analytical grade. They were used without further purification. Water was always first deionized and then distilled.

Column and column coating

A Bischoff Hyperchrome SC column (125 mm × 4.6 mm I.D.) packed with Hamilton PRP-1 was used. The particle size was 5 μm. Aqueous methyl green solution (0.5 g/l) was degassed for column coating. Potassium hydroxide was added to a pH of about 10. The methyl green was used as received without any anion exchange. The dyestuff solution was pumped through the column at a flow-rate of 0.5 ml/min until it appeared at the column exit. After completion of column coating, the system, except the column, was washed with water and afterwards with mobile phase. Then the coated column was connected with the system to obtain equilibration. If the column with sorbed dyestuff were to be washed with water, adsorbed methyl green would be removed.

Mobile phase

The organic acids were dissolved by adding potassium hydroxide in half the final volume. The pH value of this solution should be acid, otherwise difficulties arise owing to the carbonate content in the eluent. When the organic acid was completely dissolved, the required amount of methyl green was added. The eluent was degassed by a water-jet pump. The mobile phases were kept in polyethylene bottles.

Sample preparation

Samples with high contents of cations, *e.g.*, mineral water, have to be pretreated with a cation-exchange resin (Serva, Dowex 50W-X8, analytical grade, 100–200 mesh, H)[21]. An high content of carbonate can be decreased by the above pretreatment in combination with degassing of the sample. By using a 1 g/l potassium carbonate solution, it can be determined whether the cation exchanger has to be washed before use. Therefore the carbonate solution has to be pretreated as mentioned above and analyzed by the chromatographic system. With the exception of the injection and system peaks and perhaps a little carbonate peak, no peaks should appear in the chromatogram.

RESULTS AND DISCUSSION

Methyl green was chosen for the column coating on account of its quaternary ammonium group and its good water solubility. In addition, the dyestuff possesses a distinct hydrophobicity, therefore column coating can be realized. The pH value of the methyl green solution must be in the basic range during the column coating procedure, otherwise the adsorption rate will decrease due to reduced hydrophobicity of the dyestuff. The amount of dyestuff adsorbed was determined by the break-through method; 50 mg of methyl green were adsorbed on a short column as used in the present work. After column coating, the chromatographic equipment is washed with water, with the exception of the column in order to prevent loss of dyestuff. It is possible but not absolutely necessary to wash the coated column with alkaline water before equilibration with the eluent. Storing the column with the eluent is practicable for about 3 days. If the column is to be stored for a longer period, it has to be washed with methanol to remove the dyestuff, otherwise the column pressure will increase irreversibly when using the column again.

Indirect photometric detection is used for detecting "transparent" ions, as de-scribed in detail by Small and Miller[23]. The detection wavelength does not coincide with the absorption maximum of the eluent. The most suitable wavelength for the 4-hydroxybenzoic acid eluent is 311 nm; it has the most favourable signal-to-noise ratio. The wavelength was also used in preliminary studies made with a PRP-X 100
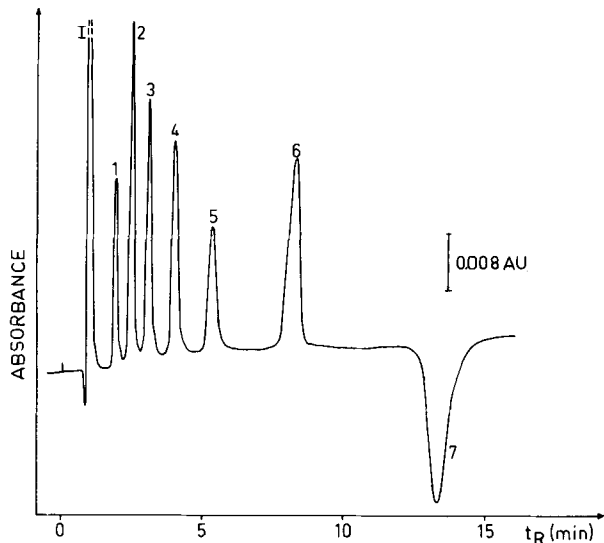

Fig. 1. Chromatogram of an anion standard solution. Eluent: $6 \cdot 10^{-3}$ mol/l 4-hydroxybenzoic acid and 50 mg/l methyl green adjusted to pH 9.0 with potassium hydroxide. Column: Bischoff Hyperchrome SC (125 mm × 4.6 mm I.D.) packed with Hamilton PRP-1 resin. The column was equilibrated with methyl green. Flow-rate: 1.0 ml/min. Sample size: 100 μl. UV detection: wavelength 311 nm. Peaks: I = injection peak; 1 = fluoride (10 mg/l); 2 = hydrogencarbonate (50 mg/l); 3 = chloride (30 mg/l); 4 = nitrite (50 mg/l); 5 = bromide (50 mg/l); 6 = sulphate (100 mg/l); 7 = system peak.

TABLE I

RETENTION OF INORGANIC ANIONS

For conditions see Fig. 1.

Anion	k'	Anion	k'
Borate	0.7	Selenite	4.7
Fluoride	1.1	Phosphate	5.4
Iodate	1.25	Arsenate	5.5
Hydrogencarbonate	1.6	Nitrate	6.5
Chloride	2.2	Sulphate	7.2
Bromate	3.1	Selenate	8.2
Nitrite	3.2	Chlorate	10.4
Bromide	4.6	System peak	12.2

chemically bonded anion-exchange resin and a mobile phase without methyl green. Methyl green does not affect UV detection at 311 nm. Preliminary studies showed that conductivity detection can also be used.

To examine the efficiency of the new column, two different eluents were chosen which had been applied to single-column anion chromatography with a chemically bonded anion-exchange resin (PRP-X 100)[21,22]. The first eluent uses 4-hydroxyben-zoic acid as the eluting agent. Fig. 1 shows the separation of an anion standard mixture obtained on a methyl green-coated column by using a 4-hydroxybenzoic acid eluent and indirect UV detection (see also Table I). The separation is similar to that achieved by Lee[22] on a chemically bonded column.

The chromatographic run time achieved with the present system was slightly longer than that reported by Lee, but a more efficient separation of fluoride from the injection peak can be realized. In addition, chloride and nitrate are baseline separated and the chromatogram shows a better peak shape. Almost the same eluent was used as proposed by Lee[22] for the separation of anions on a PRP-100 column. Only the elution power was reinforced by increasing the concentration of 4-hydroxybenzoic acid and the pH was adjusted to 9 with potassium hydroxide. The flow-rate was decreased to 1.0 ml/min, thus a smaller quantity of eluent is needed.

Methyl green was obtained as a chloride salt. If it is used in this form, interferences are observed in the detection of chloride. The chloride content in the eluent creates a small peak exactly at the elution time of chloride. The signal is positive in contrast to the chloride signal of the sample. This behaviour is in accord with results obtained with other chromatographic systems[24].

It is impossible to exchange chloride for hydroxide because the dyestuff precipitates during the exchange of the counter ion. Iodide was chosen as the counter ion because it is eluted together with the system peak. Thus the separation and detection of the other sample anions is not influenced by a resulting negative peak.

The column efficiency calculated by using the width at half peak height was 3000 plates for chloride and 4500 for sulphate with 4-hydroxybenozic acid as the eluting agent (column length 125 mm).
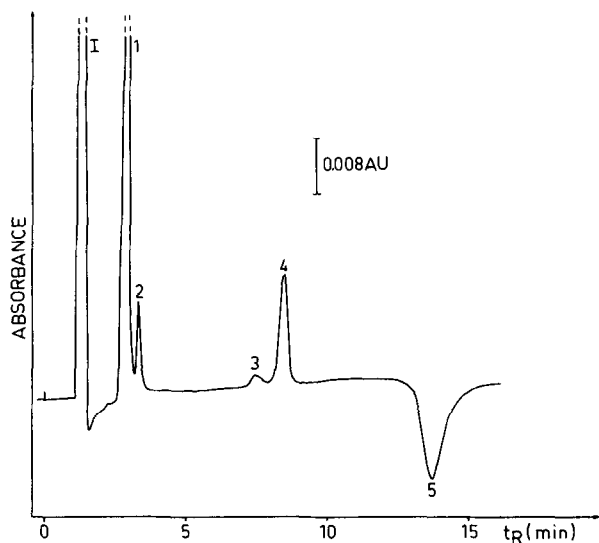
Fig. 2. Chromatogram of anions in tap-water. For conditions see Fig. 1. Peaks: I = injection peak; 1 = hydrogencarbonate; 2 = chloride (9 mg/l); 3 = nitrate (3.4 mg/l); 4 = sulphate (56.5 mg/l); 5 = system peak.

Fig. 2 shows the chromatogram of a tap-water sample. The sample is only degassed because the low content of cations does not influence the anion separation and detection. When samples of mineral water are analyzed, as illustrated in Fig. 3,
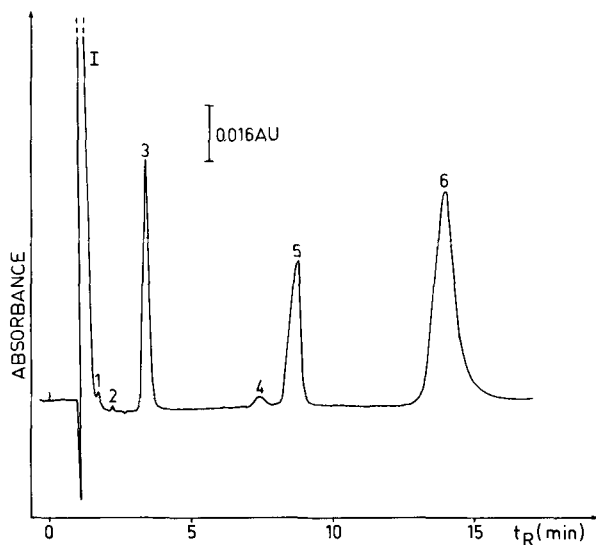

Fig. 3. Anion analysis in the mineral water "Radenska". The sample was pretreated with a cation-exchange resin and degassed (see Experimental). For other conditions see Fig. 1. Peaks: I = injection peak; 1 = borate (9.8 mg/l); 2 = fluoride (0.74 mg/l); 3 = chloride (68.3 mg/l); 4 = nitrate (5.6 mg/l); 5 = sulphate (155 mg/l); 6 = system peak.

TABLE II

DETECTION LIMITS (IN μg/l, BASED ON THREE TIMES THE BASELINE NOISE) OF SEVERAL INORGANIC ANIONS

4-Hydroxybenzoic acid (for conditions see Fig. 1)		2,4-Dihydroxybenzoic acid (for conditions see Fig. 7)	
Fluoride	100	Fluoride	20
Chloride	150	Chloride	40
Carbonate	200	Nitrite	75
Nitrite	400	Silicate	90 (as Si)
Sulphate	500	Bromide	250
Bromide	1000	Sulphate	400
		Phosphate	1500

pretreatment with a cation-exchange resin is absolutely necessary. Without this a large negative peak results from the high content of cations. Thus the detection of early eluting anions is impossible. When eluting with 4-hydroxybenzoic acid, the
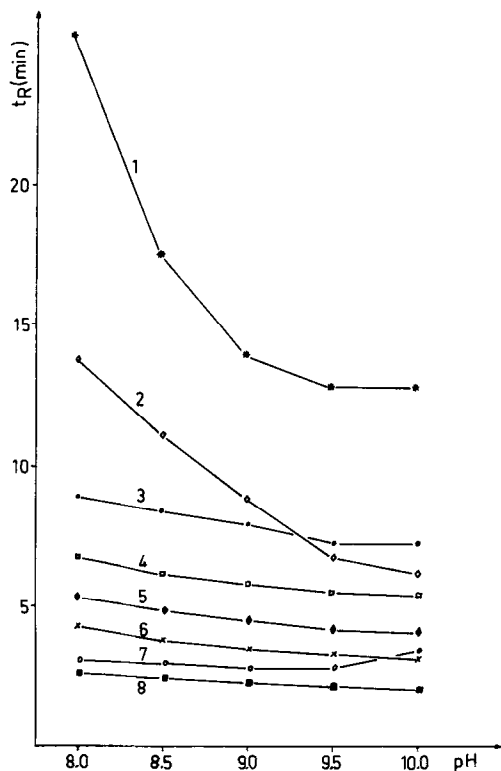

Fig. 4. Dependence of the elution time on the pH of the eluent. For other conditions see Fig. 1. 1, System peak; 2, sulphate; 3, nitrate; 4, bromide; 5, nitrite; 6, choride; 7, carbonate; 8, fluoride.

methyl green-coated column enables fluoride detection in water samples for more than 2 months, in contrast to a PRP-X 100 column that shows an evident loss of ion-exchange capacity[21].

The detection limits of six main anions are listed in Table II. They are low enough for anion analyses of natural water samples. Fig. 4 illustrates the correlation of pH and elution time of the anions. At pH < 8 no chromatogram can be obtained because of decreasing dissociation of 4-hydroxybenzoic acid. In the range pH 9.5–10.0 the baseline worsens and at pH 9.2–9.3 no separation of nitrate and sulphate can be realized. The most efficient separations were obtained at pH 9.0.

Concentrations of 4-hydroxybenzoic acid from $0.4 \cdot 10^{-2}$ to 10^{-2} mol/l were studied. As expected, higher contents of organic acid shorten the elution time. At the lowest concentration no sulphate peak appears after 40 min.

Different concentrations of methyl green in the eluent (5–100 mg/l) were tested to determine the dependence of retention on dyestuff concentration. The elution time of the anions remains nearly constant in the range of 40–100 mg/l. At dyestuff contents higher than 50 mg/l the baseline noise increases. If no dyestuff is dissolved in the mobile phase, the elution time will decrease very slowly. After 6 h, sulphate is still eluted at 6.5 instead of 8.6 min.
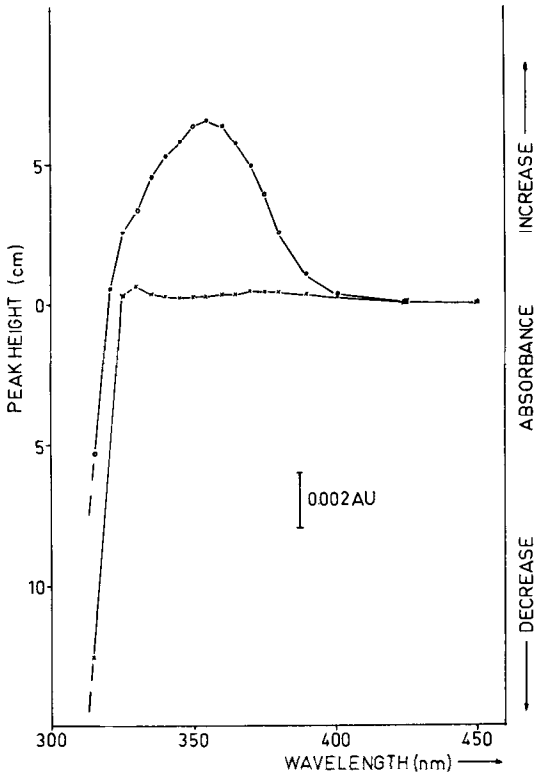

Fig. 5. Dependence of the peak height and absorbance on the detection wavelength. ○, Nitrite (50 mg/l); ×, chloride (30 mg/l). For other conditions see Fig. 1.
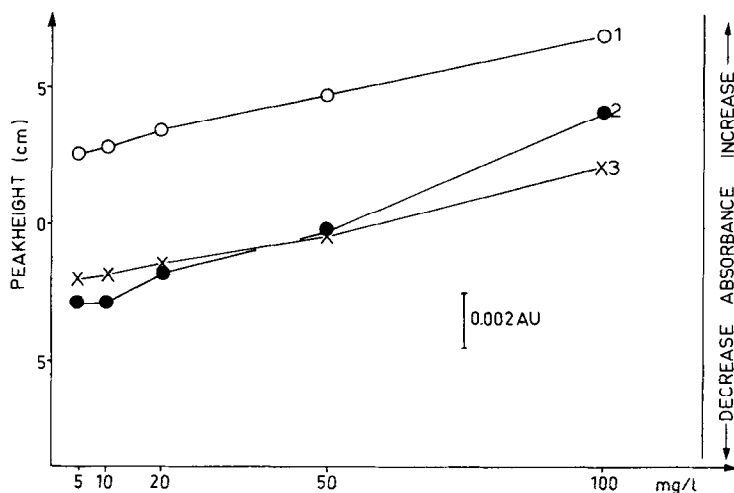
Fig. 6. Dependence of the peak height and absorbance on the methyl green concentration in the mobile phase. Detection wavelength: 324 nm. 1, nitrite (50 mg/l); 2, carbonate (50 mg/l). 3, chloride (30 mg/l). For other conditions see Fig. 1.

Two of the anions tested interact with methyl green and produce species that influence eluent absorption. The phosphate peak cannot be quantified because it is in part positive and negative. If phosphate is dissolved in the eluent, the colour of the solution changes slowly to blue in about half an hour. Eluents with a pH > 9.3 give an uniform signal for phosphate, but the baseline noise increases as mentioned above. The other interacting anion is nitrite. It changes the colour of the solution slowly to yellow when dissolved in mobile phase. Thus the peak height of the nitrite peak is not influenced in the same way as the peaks of the other anions while changing the detection wavelength (Fig. 5). The curves for other anions are similar to that obtained for chloride. At a wavelength of about 318 319 nm the peak height is zero for nitrite, while this characteristic point is reached at 324 nm for the other anions. In contrast to the other anions, nitrite has another maximum in peak height at 355 nm. Therefore selective detection of nitrite can also be achieved when a sample contains a large quantity of chloride or other neighbouring ions.

As expected, the peak height at these wavelengths is also influenced by the concentration of methyl green in the mobile phase (Fig. 6). Studies were carried out at a constant wavelength of 324 nm, where all anions except nitrite results in only very small peaks when using the normal eluent composition. The peak height of the nitrite peak is nearly linear with respect to the dyestuff content in the eluent. As stated above, the baseline noise increases at higher methyl green concentrations.

The second eluent, chosen for testing the methyl green-coated column, uses 2,4-dihydroxybenzoic acid as the organic acid in the mobile phase, so that also the determination of silicate in water samples can be realized. The concentration and pH value are the same as in previous studies with this eluent on a PRP-X 100 column[21]. The concentration of methyl green in the eluent can be decreased to 10 mg/l due to the stronger hydrophobicity of the dyestuff at higher pH.
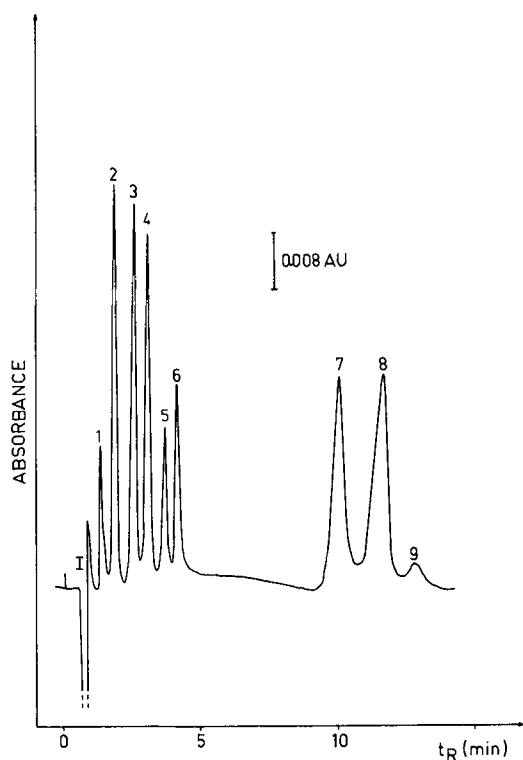
Fig. 7. Chromatogram of an anion standard solution. Eluent: 70 mg/l 2,4-dihydroxybenzoic acid and 10 mg/l methyl green adjusted to pH 10.1 with potassium hydroxide. Column: Bischoff Hyperchrome SC (125 mm × 4.6 mm I.D.) packed with Hamilton PRP-1 resin. The column was coated with methyl green. Flow-rate: 1.5 ml/min. Sample size: 100 μl. UV detection: wavelength 312 nm. Peaks: I = injection peak; 1 = silicate (10 mg/l); 2 = fluoride (3 mg/l); 3 = chloride (7 mg/l); 4 = nitrite (10 mg/l); 5 = bromide (10 mg/l); 6 = carbonate system peak; 7 = phosphate (20 mg/l); 8 = sulphate (20 mg/l); 9 = system peak.

Fig. 7 shows the chromatogram of an anion standard sample. The total elution time until the system peak has passed the detector is shortened in comparison with the separation obtained on a PRP-X 100 column. In addition, the flow-rate of the mobile phase is decreased to 1.5 ml/min, which is in part caused by the use of a shorter column. The difficulties previously described[21] with a negative peak in front of the sulphate peak at pH > 10.2 can be avoided.

Anion separation of a tap-water sample can be realized (Fig. 8). The detection limits of the main anions are listed in Table II. They are inferior to those obtained by this eluent (without methyl green) on a PRP-X 100 column[21] because of a better peak shape. The column efficiency expressed by the number of theoretical plates is 3500 for chloride and 5000 for sulphate (column length 125 mm).

The carbonate system peak results from the carbonate content in the sample and carbonate decontamination of the eluent, a fact that can be explained by the basic pH of the mobile phase.
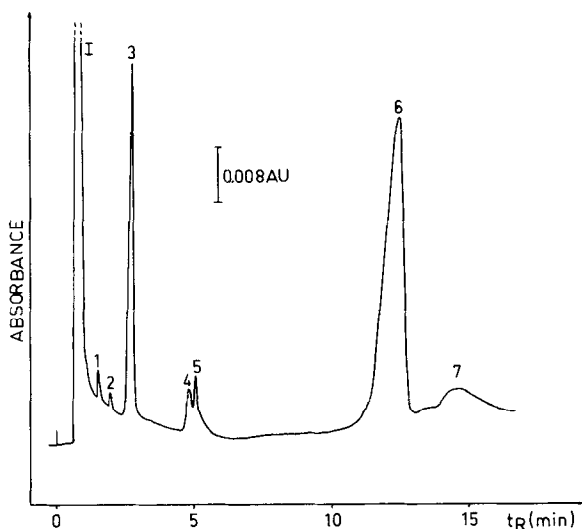
Fig. 8. Chromatogram of anions in tap-water. The sample was pretreated with a cation-exchange resin and degassed (see Experimental). For other conditions see Fig. 7. Peaks: I = injection peak, 1 = silicate (1.95 mg/l); 2 = fluoride (90 μg/l); 3 = chloride (5.8 mg/l); 4 = carbonate system peak; 5 = nitrate (3.2 mg/l); 6 = sulphate (45.6 mg/l); 7 = system peak.

It is possible to create an efficient anion-exchange column by sorbing methyl green on a styrene–divinylbenzene column. The separation system shows an increased stability compared to a PRP-X 100 column. Sorption of other organic molecules with different functional groups besides other triphenylmethane dyestuffs[25] should be possible.

ACKNOWLEDGEMENT

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